

Correlated evolution between host immunity and parasite life histories in primates and oxyurid parasites

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Maturation time is a pivotal life-history trait of parasitic nematodes, determining adult body size, as well as daily and total fecundity. Recent theoretical work has emphasized the influence of pre-maturational mortality on the optimal values of age and size at maturity in nematodes. Eosinophils are a family of white blood cells often associated with infections by parasitic nematodes. Although the role of eosinophils in nematode resistance is controversial, recent work has suggested that the action of these immune effectors might be limited to the larval stages of the parasite. If eosinophils act on larval survival, one might predict, in line with theoretical models, that nematode species living in hosts with large eosinophil numbers should show reduced age and size at maturity. We tested this prediction using the association between the pinworms (Oxyuridae, Nematoda) and their primate hosts. Pinworms are highly host specific and are expected to be involved in a coevolutionary process with their hosts. We found that the body size of female parasites was negatively correlated with eosinophil concentration, whereas the concentration of two other leucocyte families—neutrophils and lymphocytes—was unrelated to female body size. Egg size of parasites also decreased with host eosinophil concentration, independently of female size. Male body size was unrelated to host immune parameters. Primates with the highest immune defence, therefore, harbour small female pinworms laying small eggs. These results are in agreement with theoretical expectations and suggest that life histories of oxyurid parasites covary with the immune defence of their hosts. Our findings illustrate the potential for host immune defence as a factor driving parasite life-history evolution.

Keywords: age at maturity; body size; eosinophils; immune defences; pinworms

1. INTRODUCTION

The evolution of parasite life-history traits has attracted considerable attention in recent years (Poulin 1995; Sorci *et al.* 1997; Stear *et al.* 1999). This interest is partly due to the possible consequences of the understanding of parasite evolution for the management of pathogen populations and virulence (Ebert 1998; Skorping & Read 1998; Stearns 1998).

As for free-living organisms, age at maturity, fecundity and pre-maturational and post-maturational mortality are the major fitness components of parasitic nematodes (Skorping *et al.* 1991; Morand 1996; Morand & Sorci 1998). Adult female body size of nematodes is tightly linked to reproductive output, larger females having higher fecundity (Skorping *et al.* 1991; Morand 1996). Since growth stops, or sharply declines after maturation, age and size at first reproduction are usually positively correlated and are major determinants of reproductive output (Skorping *et al.* 1991; Morand 1996). Delayed maturation might, therefore, be selected for because of the fecundity

benefit of larger size at maturity. Nevertheless, delayed maturity also entails costs in terms of increased pre-maturational mortality risks; therefore, the trade-off between the fecundity benefits of delayed maturity and the mortality costs should ultimately determine the optimal age and size at maturity (Gemmil *et al.* 1999; Morand & Poulin 2000). Optimality models have explored this trade-off and predicted that age at maturity should be inversely proportional to the pre-maturational mortality rate (Gemmil *et al.* 1999; Morand & Poulin 2000). One would thus expect that nematodes with low pre-maturational mortality risks should delay maturation, grow for longer and have higher fecundity, whereas parasites with high pre-maturational mortality risks should reproduce earlier and at a smaller size.

Broadly speaking, the evolution of life-history traits is driven by the selection pressures determined by the environment where organisms live (Roff 1992; Stearns 1992). Most macroparasites experience two types of environment: the within-host environment and the external environment encountered by infective stages during the between-host transmission. Within the host, the parasite has to establish itself, grow and reproduce. These performances are affected by several environmental factors

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such as the amount of available resources or the host immune response. Hosts have evolved effective weapons against parasites, and the immune system is one of these defences (Wakelin 1996). Several studies have stressed the role played by the immune system in controlling helminth populations (Maizels *et al.* 1993; Stear *et al.* 1996, 1999; Meeusen & Balic 2000). For instance, increases in the numbers of mast cells and eosinophils are commonly observed in response to helminth infections (Maizels *et al.* 1993). Further work has shown an *in vitro* eosinophil-mediated killing of nematode parasites, although it has been more difficult to establish an *in vivo* direct effect of eosinophilia on resistance (Meeusen & Balic 2000; Viney 2002). A recent review of studies on several host–helminth associations suggested that evidence is consistent with a role for eosinophils in killing larval stages rather than adult worms (Meeusen & Balic 2000). In other words, eosinophils might act as an age-dependent mortality factor for parasites and consequently, one might expect that parasites living in a host environment with high larval mortality should reduce their age at maturation and have a smaller adult size.

We tested this prediction using the association between pinworms and their primate hosts. Pinworms are common parasitic nematodes inhabiting the caecum and proximal colon (Anderson 2000) and are particularly suitable for this study for several reasons: (i) they are highly host-specific (Brooks & Glen 1982; Hugot 1999); (ii) they almost lack a free-living stage, since hosts become infected from ingested eggs (Anderson 2000); (iii) infections with pinworm larvae in humans have been associated with blood eosinophilia, eosinophilic entero and ileocolitis (Liu *et al.* 1995; Cacopardo *et al.* 1997); (iv) reproductive output (number of eggs) is tightly correlated with body size (Cho *et al.* 1985).

We investigated patterns of correlated evolution between the sizes of females, males and eggs of 32 pinworm species and the basal values of blood circulating lymphocytes, neutrophils and eosinophils of their primate hosts. If the immune system of the hosts characterizes the environment experienced by the parasite, one might expect that high host investment into immune defence, in particular basal level of circulating eosinophils, should induce a correlated evolution towards smaller parasite size because of the benefits for the parasite to reduce age at maturity.

2. MATERIAL AND METHODS

(a) Data

Data on female size, male size and egg size (length in μm) of pinworms were extracted from Skrjabin *et al.* (1960). Data on basal levels of circulating lymphocytes, neutrophils and eosinophils (number of cells $\times 10^9 \text{ l}^{-1}$ of blood) of primates were mainly obtained from the physiological data reference values project of the International Species Information System (ISIS). These data have already been used in various studies investigating the effect of ecological factors on the evolution of primate immunity (Nunn *et al.* 2000; Nunn 2002). The complete dataset is available upon request from the corresponding author.

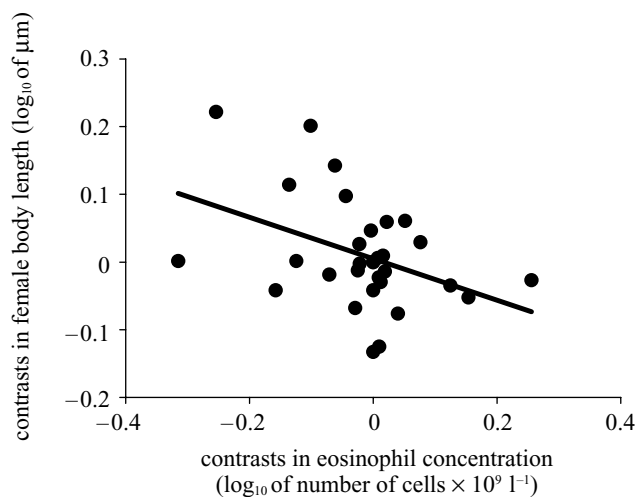


Figure 1. Negative correlation between female body length of pinworms and eosinophil concentration of primate hosts. Each point represents a phylogenetic independent contrast.

(b) Statistical analyses

Species share common ancestors and therefore cannot be considered as independent statistical units. To take into account relatedness between species we used phylogenetic independent contrasts (Felsenstein 1985; Harvey & Pagel 1991). Contrasts were computed using the CAIC statistical package (Purvis & Rambaut 1995) and the phylogenetic relationship described by Hugot (1999) with equal branch length. Phylogenetic independent contrasts were analysed with linear multiple regression models forced through the origin. Host body mass, a potential confounding variable because of correlations with cell counts and parasite size (Harvey *et al.* 1987; Harvey & Keymer 1991), was always included in the regression model. All variables were \log_{10} -transformed prior to analyses.

3. RESULTS

A stepwise multiple regression model with pinworm female body size as dependent variable and basal values of lymphocytes, neutrophils, eosinophils and host body mass as independent variables revealed that only eosinophil concentration entered the model. Primate hosts with high basal eosinophil concentration harboured smaller female pinworms (slope \pm s.e. = -0.314 ± 0.122 , $r = 0.42$, $n = 31$, $p = 0.0152$; figure 1).

A similar regression model was run for male parasite body size. However, in this case none of the independent variables entered the model (all $p > 0.2$).

Parasite egg size tended to be positively correlated with female parasite body length, although not significantly (slope \pm s.e. = 0.177 ± 0.099 , $n = 30$, $p = 0.085$). However, when running a stepwise multiple regression model with the immune variables, host body mass and female parasite body size, only eosinophil concentration entered the model. As for female parasite size, egg size was negatively correlated with basal eosinophil concentration (slope \pm s.e. = -0.161 ± 0.067 , $r = 0.41$, $n = 30$, $p = 0.0222$; figure 2).

4. DISCUSSION

Our results show a pattern of correlated evolution between host immunity, assessed as the blood concen-

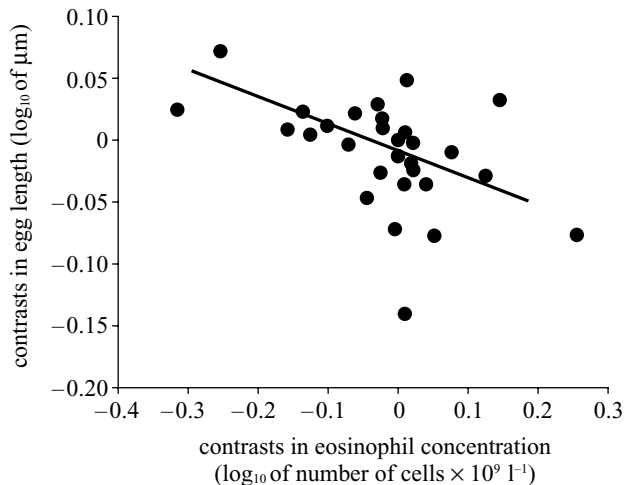


Figure 2. Negative correlation between egg size of pinworms and eosinophil concentration of primate hosts. Each point represents a phylogenetic independent contrast.

tration of eosinophils, and life-history traits of a highly specific group of parasites, the pinworms. None of the other leucocyte families (neutrophils and lymphocytes) was correlated with the adult or egg size of parasites.

The role of eosinophils in the control of helminth infections is controversial (Meeusen & Balic 2000; Dombrowicz & Capron 2001; Viney 2002). Early *in vivo* studies have shown intense infiltrates of degranulating eosinophils around damaged larvae of various helminth parasites (Moqbel 1980; Ackerman *et al.* 1990). Similarly, artificial selection experiments on mice and sheep have shown that selection for resistance to gastrointestinal parasites was associated with an increase of peripheral blood eosinophils (Dawkins *et al.* 1989; Buddle *et al.* 1992; Zhong & Dobson 1996). More recently, several studies used neutralizing anti-IL-5 and anti-IL-9 antibodies and other genetic techniques specifically to impede infection-induced eosinophilia (Herndon & Kayes 1992; Dent *et al.* 1997; Richard *et al.* 2000). This work therefore aimed to assess directly the role of eosinophils in the generation of resistance against helminths. A recent review concluded that eosinophils, together with antibody and complement, play a role in the killing of larval stages of most helminth parasites studied so far (Meeusen & Balic 2000), whereas eosinophils do not seem to have a major role in the rejection of adult worms (Meeusen & Balic 2000).

Theoretical models on life-history evolution have emphasized the role of trade-offs in determining optimal age and size at maturity (Roff 1992; Stearns 1992). Benefits of delayed maturity in terms of larger size and therefore higher fecundity could be offset if delayed reproduction increases the likelihood of prematurational mortality (Gemmil *et al.* 1999; Morand & Poulin 2000). The age-dependent effect of eosinophils on parasite survival (Meeusen & Balic 2000) and the observed negative correlation between body size of adult female pinworms and eosinophils in the peripheral blood are in agreement with these theoretical predictions on parasite life-history evolution. This result shows the potential for correlated evolution between host immune defences and parasite life histories.

Body size of adult male pinworms was unrelated to any of the immune parameters considered in this study. Pin-

worms have a haplodiploid breeding system, with high inbreeding, kin selection and female-biased sexual dimorphism (Adamson 1989; Anderson 2000). This might reduce the intensity of intrasexual competition for mates in males, relax selection on male body size and explain the lack of correlation between male size and host immunity.

Reduced age and small size at maturity might be the best options when facing elevated risks of prematurational mortality at the expense of reproductive output. Another trade-off could, however, intervene to determine the number of eggs laid. Instead of laying fewer eggs, small females might adjust the size of their eggs, maintaining a constant fecundity. This scenario might be particularly relevant for semelparous species such as pinworms (gravid females detach and move towards the anus, subsequently lay eggs in the perianal region, shrink and die; Anderson 2000). We could not test this hypothesis directly, since data on fecundity were not available. We could, however, investigate whether egg size was correlated with host immune defence. As for body size of adult females, eosinophil concentration was the only significant predictor of egg size; pinworms living in hosts with large numbers of circulating eosinophils lay smaller eggs than pinworms that exploit hosts with a lower eosinophil concentration. In the absence of data on fecundity, we cannot be sure that small females trade egg size against egg number; nevertheless, this result is suggestive of such a trade-off.

Several ecological factors have been found to correlate with leucocyte counts across primate species (Nunn *et al.* 2000; Nunn 2002; Semple *et al.* 2002). Mating promiscuity, for instance, increases the risk of sexually transmitted diseases and might select for higher investment in immune defences. Comparative tests of this hypothesis have provided supportive evidence showing that primates with a promiscuous mating system have higher baseline leucocyte counts (Nunn *et al.* 2000; Nunn 2002). Similarly, primates living in habitats characterized by high annual rainfall have higher lymphocyte and phagocyte concentrations than primates living in relatively dry habitats (Semple *et al.* 2002). Since primates suffer higher rates of parasitism in wetter habitats, the positive correlation between rainfall and investment in immune defence has been interpreted as an adaptation to combat pathogens (Semple *et al.* 2002). Overall, these studies show that a multitude of ecological factors have probably shaped the evolution of primate immunity; it is therefore unlikely that variation in eosinophil concentration, as observed in this study, directly reflects variation in pinworm prevalence and abundance. Conversely, the evolution of primate immune defences appears directly to affect life-history strategies of their pinworm parasites over evolutionary time.

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